Explicit consideration of topological and parameter uncertainty gives new insights into a well-established model of glycolysis

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Abstract

Previous models of glycolysis in the sleeping sickness parasite *Trypanosoma brucei* assumed that the core part of glycolysis in this unicellular parasite is tightly compartimentalized within an organelle, the glycosome, which had earlier been shown to contain most of the glycolytic enzymes. The glycosomes were assumed to be largely impermeable, and exchange of metabolites between the cytosol and the glycosome was assumed to be regulated by specific transporters in the glycosomal membrane. This tight compartmentalization was considered essential for parasite viability. Recently, size-specific metabolite pores were discovered in the membrane of glycosomes. These channels are proposed to allow smaller metabolites to diffuse across the membrane but not larger ones. In light of this new finding, we reanalysed the model taking into account uncertainty about the topology of the metabolic system in *Trypanosoma brucei*, as well as uncertainty about the values of all parameters of individual enzymatic reactions. Our analysis shows that these newly discovered nonspecific pores are not necessarily incompatible with our current knowledge of the glycosomal metabolic system, provided that the known cytosolic activities of the glycosomal enzymes play an important role in the regulation of glycolytic fluxes and the concentration of metabolic intermediates of the pathway.

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Supporting information

Supplementary Doc: Detailed description of the added and modified rate equations, and the sampling of the parameters.
Fig S1: Glucose consumption flux in models without any activity of the glycolytic enzymes in the cytosol.
Fig S2: Glucose consumption flux in the impermeable model (Model 1a, no activity of the glycolytic enzymes in the cytosol) when the rate of the glycerol transporter is increased.
Fig S3: Examples of comparison between the distribution of parameter values as sampled and the same parameter in the 1% best parameter sets.
Fig S4: Distributions of the percentages of activity of the glycolytic enzymes in the cytosol among the top 1% of parameter sets of models 1a, 3 and 4.
Fig S5: Percentages of parameter sets allowing each model topology to reach steady-state before 1000 simulated minutes.
Table S1: Parameters significantly different in the best parameter sets (highest 1% log-likelihood) when compared to the other 99% sampled values.

Keywords

Computational modelling; systems biology; glycolysis; parameter sampling; topological uncertainty

Introduction

The kinetic model of glycolysis in the sleeping sickness parasite *Trypanosoma brucei* was one of the first detailed kinetic models of metabolism for which measured enzyme kinetic parameters were used, rather than presumed [1]. The model postulated that the core part of glycolysis in this unicellular parasite is tightly compartmentalized within an organelle, the glycosome, which had earlier been shown to contain most of the glycolytic enzymes [2]. The glycosome was considered to be largely impermeable. Exchange of metabolites was assumed to be regulated by specific transporters in the glycosomal membrane. This assumption was supported by the experimentally validated model prediction that mislocalisation of glycolytic enzymes to the cytosol leads to a lethal accumulation of sugar phosphates in the cytosol [3, 4]. This encouraging agreement between modelling and experiment reinforced the conviction that leaky glycosomes would be incompatible with parasite physiology. Notwithstanding, arguments that the glycosome membrane should be considered only as semi-permeable were made [5].

Recently, size-specific metabolite pores were discovered in the membrane of the glycosomes [6]. These channels are proposed to allow smaller metabolites to diffuse freely across the membrane, but not larger ones. This observation adds support to the idea that glycosomes are not impermeable. In this paper, we show that these newly discovered nonspecific pores are not necessarily incompatible with our current knowledge of the glycosomal metabolic system provided that the known *cytosolic* activities of the glycosomal enzymes play an important role in the regulation of glycolytic fluxes and the concentration of metabolic intermediates of the pathway.

We do so by taking into account uncertainty about the topology of the metabolic system in *T. brucei*, as well as uncertainty about the values of all parameters of the enzymatic reactions [7]. Comparing different model topologies and taking parameter uncertainty into account reveals unexpected robustness to glycosome leakiness. The model also uncovers several major gaps in our current understanding of trypanosome glycolysis which should guide further experimentation towards a full description of this pathway in *T. brucei*.

The 14 different models are available in SBML format, with a tab-delimited file including 100,000 parameter sets as they were sampled, at https://seek.sysmo-db.org/models/107.

Results and Discussion

A few methods for dealing with this uncertainty have been suggested before [8, 9, 10, 11, 12]. In this context, we have previously analysed the existing model of *T. brucei* glycolysis taking into account uncertainty about the enzyme parameter values, but keeping the topology of the model fixed [7]. In addition, we incorporate a second tier of uncertainty into

the model with regard to its topology, i.e., considering which reactions occur, and in which subcellular compartment. Topological uncertainty is regularly dealt with in the context of genome-scale metabolic reconstructions (see for example [13]), but is usually disregarded in dynamic model analysis. For this purpose, we constructed alternative model versions with different topologies. We then simulated each model version using a large range of plausible parameter sets which were sampled from distributions reflecting our degree of uncertainty about each parameter (see [7] and Methods for details).

Exploring the effect of a permeable glycosome

In the original model published in 1997 and in all of its subsequent iterations [1, 14, 15, 3, 16, 17, 7], the glycosomes have been considered to be impermeable (see Fig. 1). However, the recently discovered pores [6] would allow free diffusion of the smaller metabolites between the cytosolic and the glycosomal lumen. This has been considered to be incompatible with the survival of the parasite, due to the accumulation of sugar phosphates observed when the glycosomal enzymes are mislocalised to the cytosol and therefore exposed to a high ATP/ADP ratio. This phenomenon, referred to as a “turbo explosion”, is assumed to result from the loss of specific regulatory feedbacks for critical enzymes [3, 4]. Consequently, after the discovery of unspecific pores, mechanisms that would nonetheless permit all metabolites to be retained inside the glycosomes have been hypothetised, but no evidence for any of them has been found [18]. Here, we explore the consequences of increasing the permeability of glycosomes in a series of alternative computational models and reveal an important role for residual cytosolic activities of several glycolytic enzymes whose function had hitherto been unclear.

To represent our uncertainty about the permeability of the glycosome, we constructed different versions of the model in which different groups of metabolites can freely diffuse across the glycosomal membrane. We defined the different groups of metabolites based on their molecular weight (see Figure 2). Based on these groups and on the last published version of the model [7] (which we updated by explicitly including glycerol transporters, see Methods and Supplementary Doc S1 for details), we built seven different model versions, each permitting an increasingly large set of metabolites to diffuse freely.

Among these seven models, we included two versions of the original impermeable model: model 1a, in which the transport of glycerol 3-phosphate and dihydroxyacetone phosphate is linked via an antiporter in the glycosomal membrane, as introduced in 1999 [14], and model 1b, in which the transport of glycerol 3-phosphate and dihydroxyacetone phosphate is independent, as was the case in the first published version of the model in 1997 [1]. The glycerol 3-phosphate/dihydroxyacetone phosphate antiporter was introduced to lower the effect of the inhibition of the glucose consumption flux by glycerol, thus allowing a better agreement of the model with experimental results [14]. Therefore, we expect model 1a (with antiporter) to perform better than model 1b (without). Experimental evidence for the antiporter is lacking. However, the requirement for such a transporter disappears if glycerol 3-phosphate and dihydroxyacetone phosphate are able to diffuse using the newly identified permeability pores. Therefore, it is important to evaluate the effect on model behaviour of the replacement of the specific antiporter by diffusion pores.

Since exposing the glycosomal enzymes to the ATP/ADP ratio of the cytosol leads to a "turbo explosion" when cells are exposed to high concentrations of external glucose [4], we know that at least model 6 (which allows ATP and ADP to diffuse freely) should show this lethal increase in sugar-phosphate concentrations when the external glucose concentration is high. We simulated all model versions at both 5 and 50 mM of glucose in the medium and in both aerobic and anaerobic-like conditions. In our model, the anaerobic case was simulated by including an inhibitor (Salicylhydroxamic Acid or SHAM) of the trypanosomal glycerophosphate oxidase system (GPO, the only oxygen-using reaction in the model which covers two reactions *in vivo*: glycerol 3-phosphate and trypanosome alternative oxidase), in order for our simulation process to remain as close to the experimental setting as possible (see Supplementary Doc S1 for details).

Under aerobic conditions (see Figure 3 A and B), most models lead to similar predictions for the glucose consumption flux. Only model 4 (in which glycosomes are permeable to all metabolites other than AMP, ADP, ATP, NAD and NADH) and model 5 (similar to model 4, but where glycosomes are also permeable to AMP) show lower fluxes for some parameter sets. It has been shown that the parasite dies when the glucose consumption flux is inhibited more than 50% [19], therefore models 4 and 5 are not compatible with experimental data for some parameter sets. In anaerobic conditions, however, all models show lower glucose consumption fluxes than in aerobic conditions, except for model 6 (in which glycosomes are permeable to ADP and ATP). Models 1b, 2, 3, 4 and 5 (which are models with increasingly permeable glycosomes) show significantly lower fluxes than model 1a (the impermeable model with a glycerol 3-phosphate/dihydroxyacetone phosphate antiporter). Hence, the absence of the antiporter is sufficient to render the performance of the impermeable model as poor as the semi-permeable models (models 2 to 5). Model 6 is the only one to show a similar distribution of glucose consumption fluxes in all conditions (see figure 3), which is consistent with experimental data [20, 15]. However, model 6 shows a dramatic increase in the intracellular sugar phosphate concentrations, especially at higher glucose concentrations, corresponding to the expected "turbo explosion" phenomenon which would be lethal *in vivo* (see the example of fructose 1,6-bisphosphate concentration in Figure 4), hence model 6 is not a realistic description of intact trypanosomes, as expected.

From these results, the model in which the glycosome is impermeable with a glycerol 3-phosphate/dihydroxyacetone phosphate antiporter (model 1a), allows the best match between experimental results and simulations. However, the discovery of non-specific metabolite pores in the glycosomal membrane and the absence of experimental evidence for the antiporter means that a dilemma remains. Moreover, model 1a is inconsistent with several other facts. Most importantly, all parameter sets result in a lower glucose consumption flux in anaerobic conditions than in aerobic conditions, which is contrary to experimental observations [20, 15]. Model 1a did not show any decrease in glucose consumption flux in anaerobic conditions in previous versions of the model. Indeed, in earlier versions, the glycerol concentration was fixed to zero inside the glycosome, which means that the transport of glycerol outside was considered infinitely fast and the glycerol concentration outside as infinitely low. Both of these assumptions are unrealistic. When introducing the glycerol transporter, even considering that the transport can favour glycerol

export over import and setting its V_{max} up to more than twice as fast as the measured V_{max}, some inhibition of glucose consumption already appears with a very small concentration of glycerol outside the cell (see Supplementary Figure S1). Even when the glycerol transporter V_{max} is increased to much higher values (up to 1000 nmol/min/mg of cell protein, which is 10 times the rate of the glucose transporter), glucose consumption is still predicted to be lower in anaerobic conditions (see Supplementary Figure S2).

In addition, model 1a neglects the need for glucose 6-phosphate and fructose 6-phosphate in the cytosol. Indeed, previous versions of the model also assumed that the glycosomal enzymes have no activity in the cytosol. However, several glycolytic metabolites are required in the cytosol as input to other pathways, for example, glucose 6-phosphate for the cytosolic branch of the pentose phosphate pathway [21] or fructose 6-phosphate for the synthesis of fructose 2,6-bisphosphate [22]. These metabolites either come from the glycosome or are synthesised directly in the cytosol, implying the presence of some glycolytic enzymes in the cytosol. Moreover, it has been known for many years that specific isoforms are present for key enzymes including glyceraldehyde phosphate dehydrogenase (GAPDH). The glycosomal enzymes are imported fully folded and functional [23, 24]. Therefore, even when specific cytosolic isoforms have not been identified, activity of the freshly synthesized enzymes, prior to their entry into the glycosomes, will be found in the cytosol.

Exploring the effect of specific or residual activity of the glycosomal enzymes in the cytosol

Uncertainty pertaining to the topology of the model means that some fraction of activity of the glycosomal enzymes in the cytosol should be included. Data about the percentage of activity in the cytosol is very limited except for glyceraldehyde-3-phosphate dehydrogenase, which is present in the cytosol as the product of a different gene than that encoding the glycosomal version (see Supplementary Doc S1). Although measurements of these fractions have been attempted [2, 25], the fragility of glycosomes and the properties of some enzymes (such as the tendency of hexokinase to stick to membranes [26]) have precluded any reliable quantification. We assumed, however, that the percentage of cytosolic activity of these enzymes is probably small, and certainly more than 50 percent of each of these enzymes is in the glycosomes. Therefore, percentages were sampled from a log uniform distribution between 0.01% and 50% (using a log uniform distribution allows each order of magnitude to be sampled in similar proportions, and also ensures that the unlikely very high values are only rarely sampled; 80% of the values will be < 5%; see Supplementary Doc S1 for details).

Figure 5A and B show that most models with an explicit fraction of cytosolic activity of glycolytic enzymes have similar distributions of glucose consumption flux under aerobic conditions, with only a small proportion of some models with some sampled parameter sets showing a drop in flux that differs from experimental observations. However, under anaerobic conditions (Figure 5C and D), models 3, 4 and 5 (which have glycosomes permeable to metabolites smaller than, respectively, fructose 6-phosphate, fructose 1,6-bisphosphate and AMP) allow higher glucose consumption fluxes at both 5 and 50 mM of

glucose than when cytosolic activities of the glycolytic enzymes was not considered (Figure 3). Therefore, introducing a fraction of cytosolic activity of the glycolytic enzymes allows models with semi-permeable glycosomes to simulate experimental data, i.e. fluxes and metabolite concentrations, much better than when the cytosolic fraction is ignored. We went on to test whether these higher fluxes are always associated with lethal accumulation of sugar phosphates at 50 mM of glucose in aerobic conditions. Figure 6B shows that in both aerobic and anaerobic conditions, most parameter sets do indeed lead to an increased fructose 1,6-bisphosphate concentration compared to the same model without any glycolytic activity in the cytosol (Figure 4). As expected, model 6 still shows a dramatic increase in fructose 1,6-bisphosphate. This shows that adding cytosolic activity of the glycolytic enzymes can allow the glucose consumption flux to be higher anaerobically, but that some of these parameter sets lead to an increase of the sugar phosphate concentrations, sometimes up to several hundreds of millimolar. To better simulate experimental observation, a model should allow both higher fluxes and avoid these accumulations of sugar phosphate. Therefore, to be able to know which model is the most realistic, every model and every parameter set needs to be evaluated by comparing its simulated results with all experimental data available (metabolite concentrations and fluxes).

However, matching experimental data of this complexity to model predictions offers great challenges, since some aspects of the metabolic phenotype can match experimental results while, simultaneously, others do not. To quantify the match between the models and the experimentally measured concentrations and fluxes, we computed log-likelihoods for each model and for each parameter set. These log-likelihoods are computed by comparing the simulated values of the whole-cell metabolite concentrations and fluxes to distributions based on a wide range of experimental data. These distributions represent our (uncertain) knowledge of the intracellular metabolite concentrations, the glucose consumption flux, and the glycerol production flux relative to the pyruvate production flux in both aerobic and anaerobic conditions, at 50 mM of external glucose (which is the concentration of glucose used in the experiments measuring the intracellular metabolite concentrations; see Methods for details). The higher the log-likelihood (closer to zero), the better the match is between model prediction and experimental data.

The distributions of the log-likelihoods for each model topology are shown in Figure 7. As expected, model 6 never performs as well as the best models. Indeed, since ATP and ADP can diffuse freely in this model, the model is less sensitive to the proportion of activity of the glycosomal enzymes in the cytosol. The models that allow the highest log likelihood (Figure 7) for the largest number of parameter sets are models 3 and 4 (the models with glycosomes permeable to small metabolites up to the size of fructose 6-phosphate and fructose 1,6-bisphosphate respectively). This shows that, based on our current knowledge, permeable models can yield matches to the experimental data that are equally good as the original model that depends on an impermeable membrane.

Identification of the best parameter sets for each model topology

It is striking that no matter which topology we preferred, there is always a large fraction of perfectly plausible parameter sets that lead to highly unlikely model predictions. We aim to

exploit this observation to update our beliefs about the most likely parameter values. Using the log-likelihoods, we tested whether the top performing 1% of parameter sets for each model have significantly different parameter values compared to the other parameter sets. The results are shown in Table 1 (selected V_{max} only) and Supplementary Table S1 (see Supplementary Figure S3 for two examples and Supplementary Figure S4 for the distribution of the proportion of activities in the top 1% of parameter sets). Indeed, the best matches to experimental data tend to occur for particular choices of parameters, and some of these are similar for all model topologies. First, the phosphoglycerate mutase (PGAM) V_{max} is usually higher in the best 1% of parameter sets for all models. This is expected, since previous analyses showed that decreasing this parameter leads to an accumulation of 3-phosphoglycerate [7]. The top 1% of parameter sets for all models also tend to have glucose transporter activity close to the minimal plausible value, allowing a flux of about 100 nmol/min/mg of cell protein (see Supplementary Figure S3A). The effect of this reduction in transporter activity is to lower the concentration of free glucose in the cytosol, thus limiting the concentration of glucose 6-phosphate that would accumulate due to cytosolic hexokinase. This limited activity of the transporter is also in agreement with the finding that the glucose transporter has a significant control over the glucose consumption flux [14]. The fraction of parameter sets that allows the models to reach steady state is close to 100% for most model topologies (See Supplementary Figure S5).

Also common to all models, the glycerol transporter V_{max} is usually set to higher values in the best parameter sets, so that glycerol is rapidly exported. This relates to the best models being those whose flux in anaerobic conditions is higher, hence where the inhibition of the flux by intracellular glycerol is the lowest. Under anaerobic conditions, for one mole of glucose consumed, one mole of glycerol and one mole of pyruvate are produced. This exact stoichiometric match is due to the fact that for each glucose consumed, two ATP molecules are used in the glycosomes (by hexokinase and phosphofructokinase); this ATP needs to be recovered by phosphoglycerate kinase (pyruvate branch) and glycerol kinase (glycerol branch). However, since the glycerol kinase equilibrium strongly favours ATP consumption using glycerol as its substrate, even a little accumulated glycerol inhibits ATP production. Indeed, it has been shown that addition of glycerol under anaerobic conditions kills the parasites, while without addition of external glycerol the glucose consumption flux in aerobic and anaerobic conditions is similar [27, 28, 29]. This inhibitory effect can be reduced by the increase of glycerol export, while also increasing the fraction of glycerol kinase in the glycosome. This also explains that the glycerol kinase V_{max} is higher in the glycosome of the top 1% of parameter sets of each model.

A particularly surprising observation is the fact that models 1 to 3 favour having a higher proportion of aldolase activity in the cytosol in the best 1% of parameter sets: there seems to be no intuitive reason why the level of aldolase activity would lead to reduced accumulation of sugar-phosphates (including fructose 1,6-bisphosphate). The fraction of aldolase in the cytosol seems to be important, as long as fructose 1,6-bisphosphate cannot be transported across the glycosomal membrane. The fraction of hexokinase in the cytosol of the top 1% of parameter sets is also different depending on the model topology. In the best parameter sets of model 1a, 1b and 6 it is relatively small (the median value is about 4 nmol$\cdot$ min$^{-1}\cdot$(mg of

cell protein)$^{-1}$ for model 1a, 9 nmol· min^{-1}·(mg of cell protein)$^{-1}$ for models 1b and 6, see Supplementary Table S1) while in models 2, 3 and 5 it is higher (the median value is 40 nmol· min^{-1}·(mg of cell protein)$^{-1}$ for model 2, 53 nmol· min^{-1}·(mg of cell protein)$^{-1}$ for model 3 and 190 nmol· min^{-1}·(mg of cell protein)$^{-1}$ for model 5; model 4 shows no significant difference in cytosolic hexokinase activity between the top 1% of parameter sets and the other parameters sets: the median value of all parameter sets is about 13 nmol· min^{-1}·(mg of cell protein)$^{-1}$). However, the highest cytosolic hexokinase V_{max} values never lead to a good match with the experimental data (see Supplementary Figure S3B). Differences are also observed for phosphoglycerate kinase, which is significantly higher in the cytosol of the best parameter sets of model 1a and 3 to 5 but lower in models 2 and 6. Additionally, glyceraldehyde phosphate dehydrogenase (GAPDH) activity in the cytosol is also significantly higher in the top 1% of parameter sets of models 1a, 3 and 4. Therefore, the best model topologies (models 1a, 3 and 4) seem to favor a higher flux in the cytosolic branch of the lower part of glycolysis (producing 3-phosphoglycerate from glyceraldehyde 3-phosphate). Such a topology could allow these models to compensate for the loss of bound phosphates in the glycosome due to the the leakiness of the glycosomal membrane (model 3 and 4). This may seem inconsistent with experiments that previously showed [30] that expressing the cytosolic version of phosphoglycerate kinase in bloodstream form parasite causes cell death, even for a low level of expression. However, in those experiments no difference in metabolite concentration or flux related to glycolysis could be measured in the overexpressing lines, and a general toxicity effect could not be excluded [30].

Many other parameters are distributed differently, depending on the model, and could potentially be used to design discriminatory experiments to decide in favor of one or the other topology, should the necessary measurement technology become available. For example, the triose phosphate isomerase (TPI) V_{max} in the cytosol is significantly different for the best 1% of all models except models 2 and 5. Higher cytosolic TPI V_{max} values are favoured for model 1a (the impermeable model with antiporter) but lower values are favoured for models 1b (no antiporter) to 6. This suggests that the free diffusion of dihydroxyacetone phosphate and glycerol 3-phosphate makes models sensitive to the presence of TPI in the cytosol. It has been shown that overexpressing this enzyme in the cytosol kills the parasite. However, the overexpression was important and, as is the case for PGK, the cause of the death of the cells with overexpression of TPI was not established, and a toxic effect of the overexpression per se (in contrast to a mere mis-targeting) could not be excluded. Establishing how sensitive trypanosomes are to the expression of TPI, and other glycosomal enzymes, in the cytosol and what the associated metabolic changes are, would be valuable information to help establishing which of our models is the closest mechanistically correct representation of the biological system.

Conclusion

We have shown, using mathematical modelling, that glycosomes do not need to be impermeable to explain glycolysis in trypanosomes. There is no need to hypothesize additional mechanisms to keep metabolites inside the glycosomes if a regulated proportion of glycolytic enzyme activity is found in the cytosol. The modelling results, based on an

explicit representation of parameter and topology uncertainty, point to key experiments that will help to determine the exact level of permeability of the glycosomes.

Methods

Model

The starting point for all models in this paper is the last updated version [7] of the glycolysis model of *T. brucei* first published by Bakker *et al.* in 1997 [1]. See Supplementary Doc S1 for modifications and additions.

The 14 different models are available in SBML format, with a tab-delimited file including 100,000 parameter sets as they were sampled, at https://seek.sysmo-db.org/models/107. See Supplementary Doc S1 for details.

Sampling of parameters

The parameters already present in the previous version of the model are sampled as in [7]. See Supplementary Doc S1 for details about the modifications, the sampling of the newly introduced parameters and the simulations.

Log-Likelihood

To compute the log-likelihood of each parameter set for each model, we first defined probability distributions representing our beliefs about the whole-cell metabolite concentrations and fluxes based on experimental data. Then, for each parameter set and for each model, the predicted steady-state concentrations and fluxes are compared to these distributions to compute the log-likelihood of each parameter set given our belief about the true concentrations and fluxes. See Supplementary Doc S1 for details.

Statistical tests

The parameters of the 1% best models were compared to the other parameters sets using the Mann-Whitney U test and the false discovery rate was controlled using the Benjamini-Hochberg procedure (with $\alpha = 5\%$).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPO	Glycerol 3-phosphate oxidase system

PGAM	Phosphoglycerate mutase
PGK	Phosphoglycerate kinase
SHAM	Salicylhydroxamic Acid
TPI	Triose phosphate isomerase

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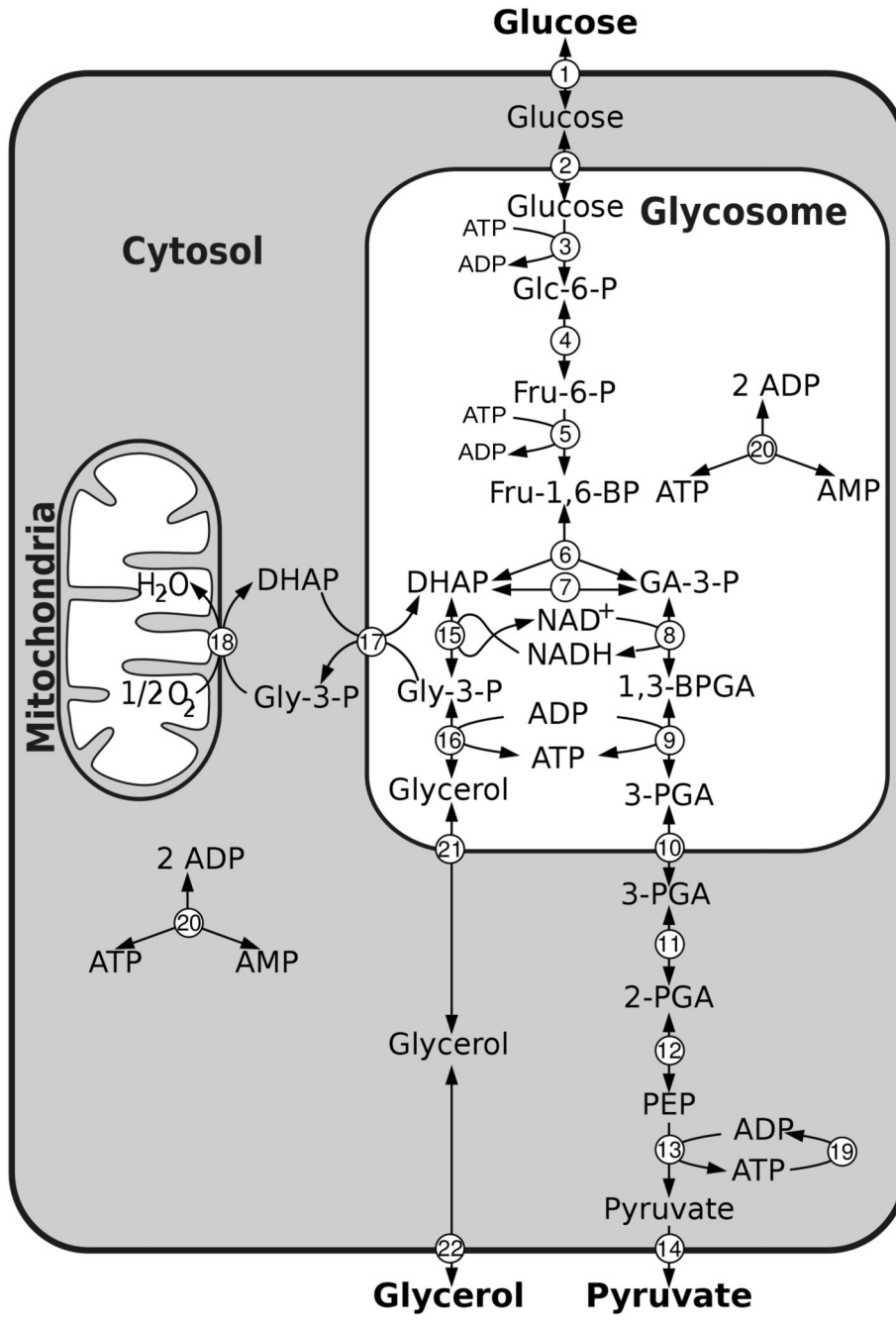

Figure 1.

Aerobic glycolysis in bloodstream form *T. brucei*. Abbreviations: Metabolites: Glc-6-P = Glucose 6-phosphate, Fru-6-P = Fructose 6-phosphate, Fru-1,6-BP = Fructose 1,6-bisphosphate, DHAP = dihydroxyacetone phosphate, GA-3-P = glyceraldehyde 3-phosphate, Gly-3-P = glycerol 3-phosphate, 1,3-BPGA = 1,3-bisphosphoglycerate, 3-PGA = 3-phosphoglycerate, 2-PGA = 2-phosphoglycerate, PEP = phosphoenolpyruvate. Reactions: 1 = transport of glucose across the cytosolic membrane, 2 = transport of glucose across the glycosomal membrane, 3 = hexokinase, 4 = phosphoglucose isomerase, 5 =

phosphofructokinase, 6 = aldolase, 7 = triosephosphate isomerase, 8 = glyceraldehyde 3-phosphate dehydrogenase, 9 = phosphoglycerate kinase, 10 = transport of 3-PGA across the glycosomal membrane, 11 = phosphoglycerate mutase, 12 = enolase, 13 = pyruvate kinase, 14 = transport of pyruvate across the cytosolic membrane, 15 = glycerol 3-phosphate dehydrogenase, 16 = glycerol kinase, 17 = DHAP-Gly-3-P antiporter, 18 = glycerol-3-phosphate oxidation, 19 = ATP utilisation, 20 = adenylate kinase, 21 = glycosomal glycerol transporter, 22 = cytosolic glycerol transporter.

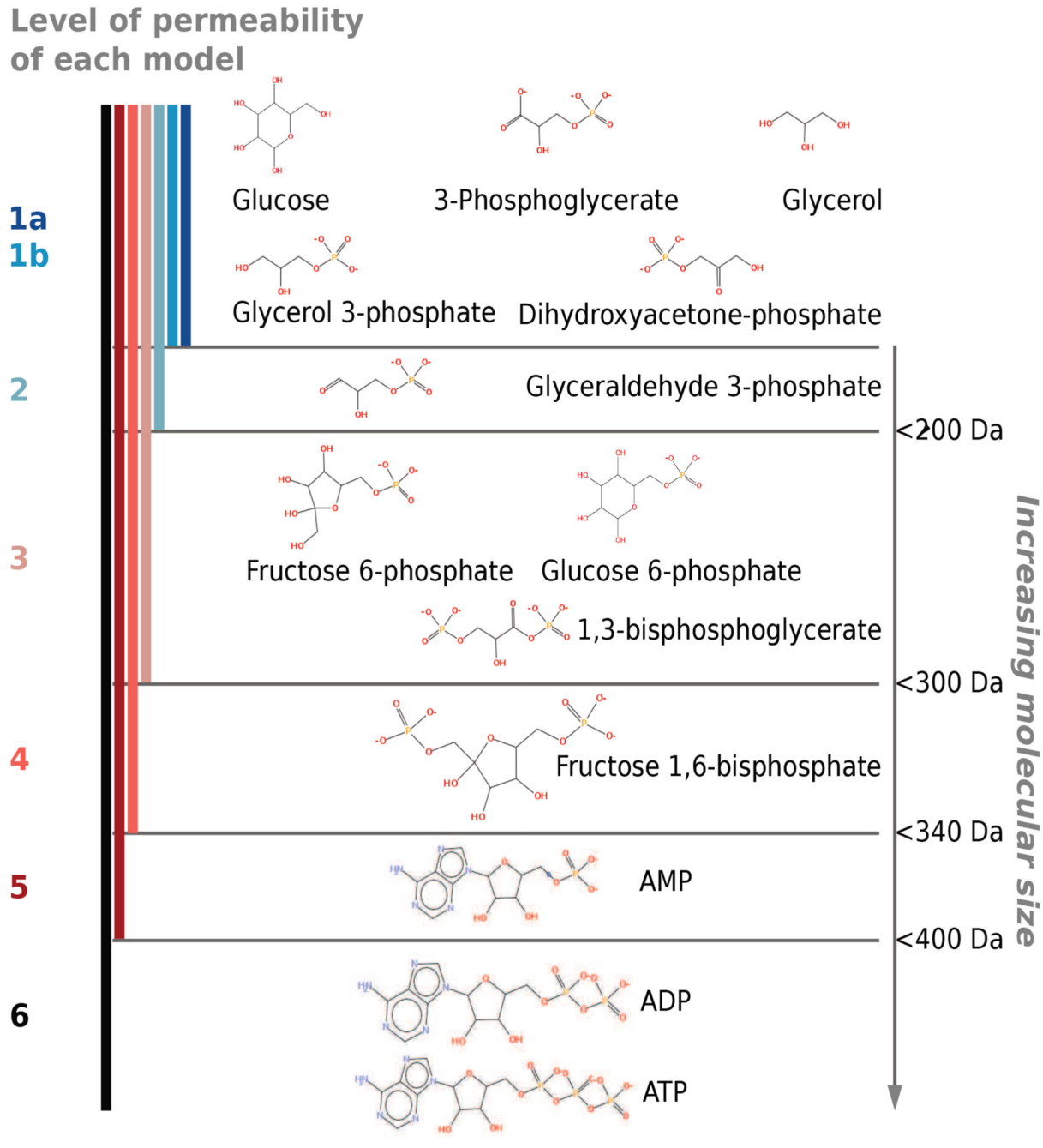

Figure 2.

Definition of the different types of models based on the size of metabolites that can be transported across the glycosomal membrane. Model 1a and 1b differ by the nature of the transport of glycerol 3-phosphate and dihydroxyacetone phosphate. In model 1a, their transport is linked via an antiporter, as introduced in 1999 [14], while in model 1b they are transported independently, as was the case in the original model of 1997 [1].

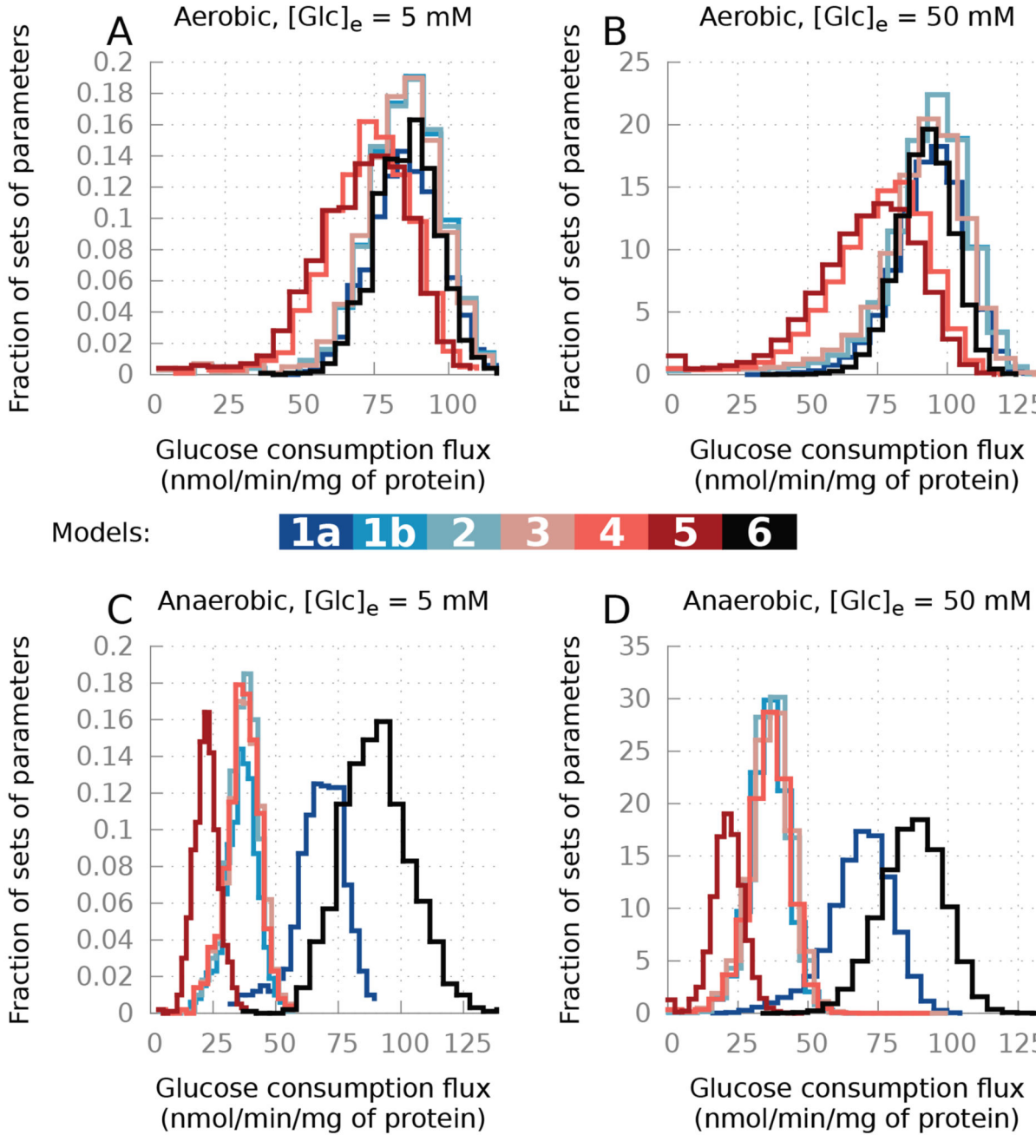

Figure 3.

Glucose consumption flux in models with increasing glycosome permeability. (A, B) Aerobic conditions (−SHAM); (C,D) Anaerobic-like conditions (+SHAM). (A, C) 5 mM of external glucose; (B,D) 50 mM of external glucose. The glucose consumption flux is lower in anaerobic conditions than in aerobic conditions for all models, and more so for the permeable models, which is in contradiction with experimental results.

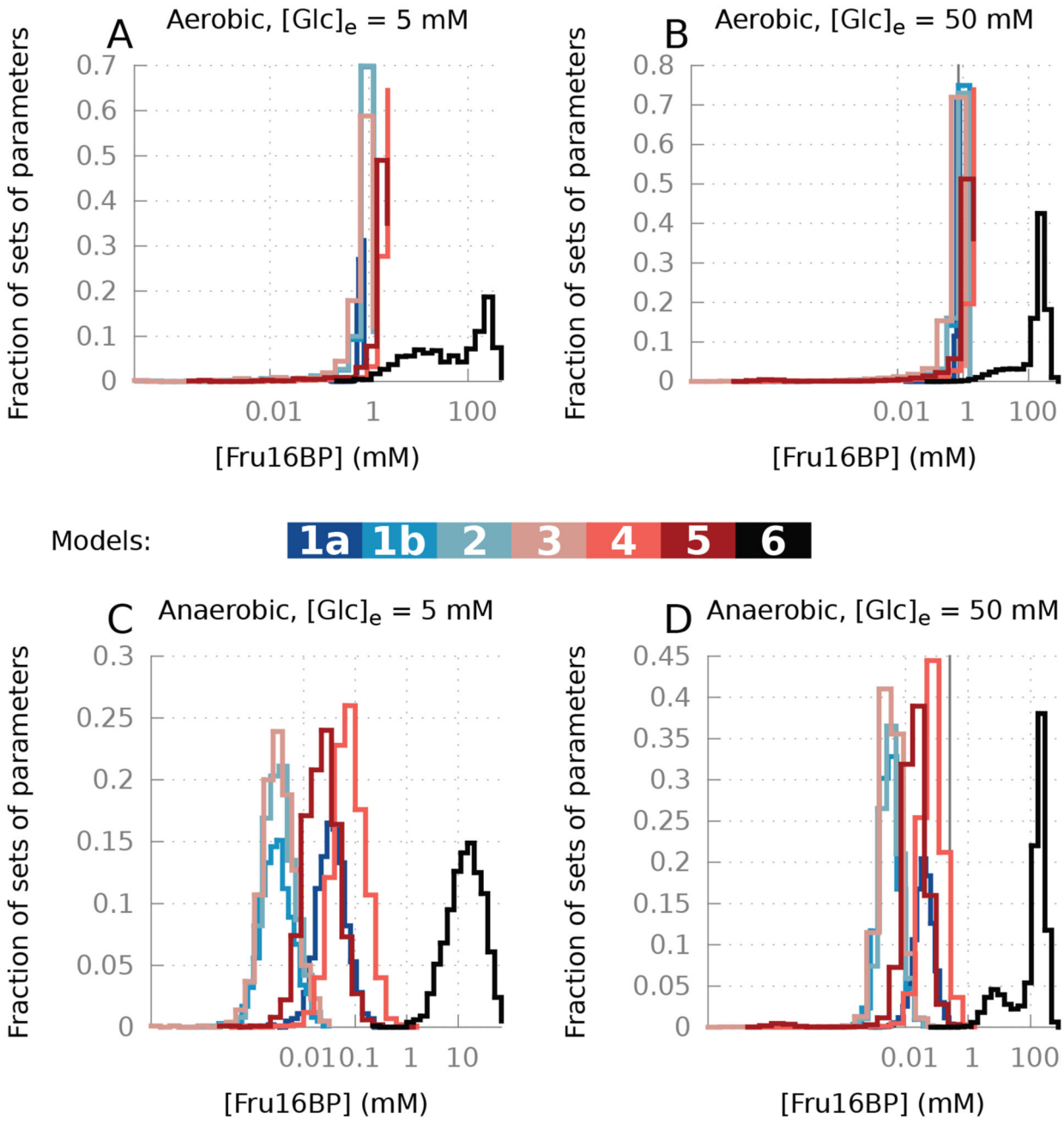

Figure 4.

Fructose 1,6-biphosphate concentration in models with increasing glycosome permeability. (A, B) Aerobic conditions (−SHAM) (C,D) Anaerobic-like conditions (+SHAM) (A, C) 5 mM of external glucose, (B,D) 50 mM of external glucose; the vertical grey line indicate the average concentration of fructose 1,6-bisphosphate as measured by Visser and Opperdoes *et al.* [31]. All models maintain realistic concentrations except model 6 which accumulated fructose 1,6-bisphosphate in all conditions, but more importantly at 50 mM of external glucose.

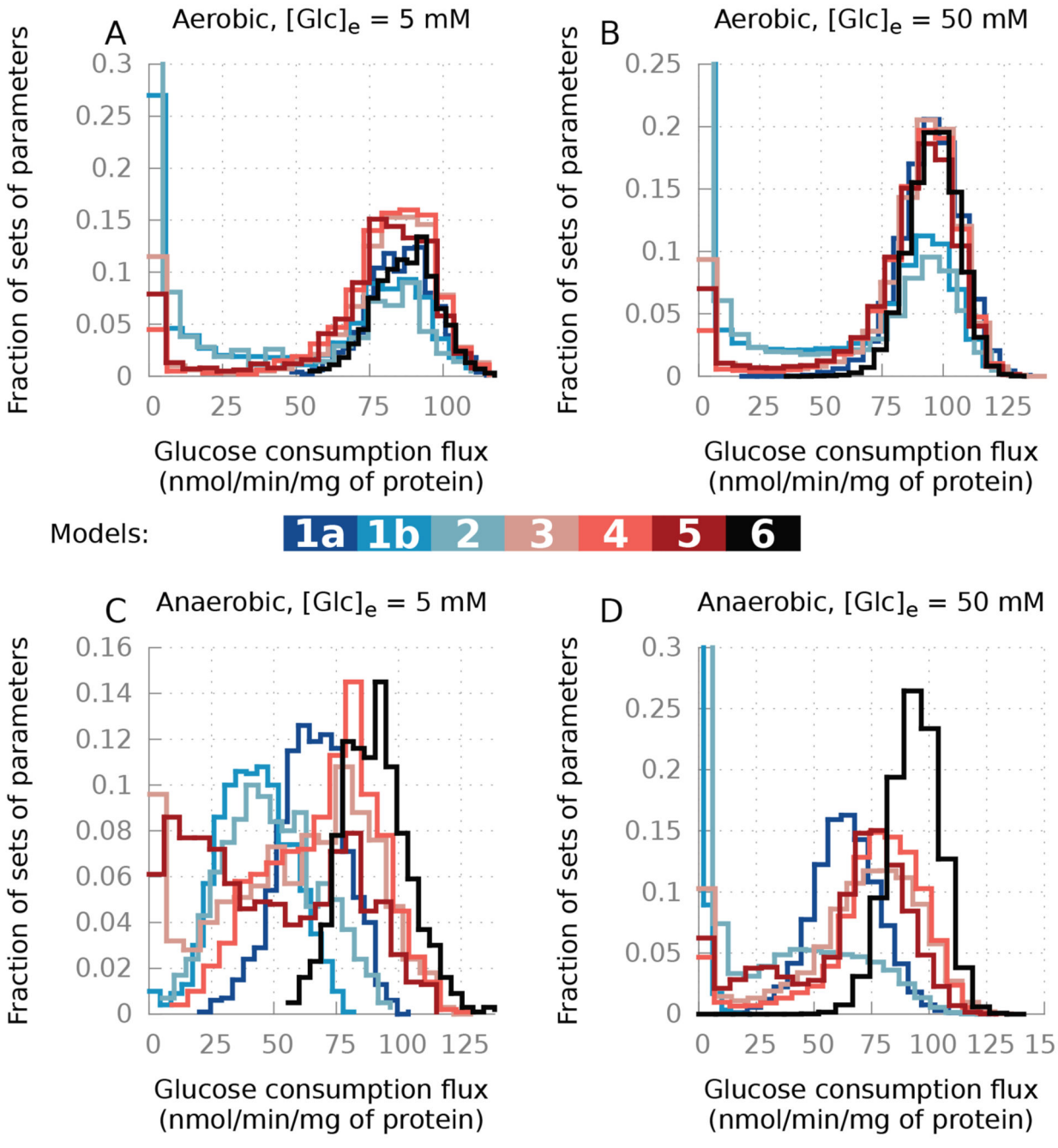

Figure 5.

Glucose consumption flux in models with increasing glycosome permeability and cytosolic activities of the glycosomal enzymes. The distributions shown include all sampled fractions of cytocolic activities of the glycosomal enzymes for each model topology. (A, B) Aerobic conditions (−SHAM) (C,D) Anaerobic-like conditions (+SHAM) (A, C) 5 mM of external glucose, (B,D) 50 mM of external glucose. More models allow a similar flux in both aerobic and anaerobic conditions when a fraction of cytosolic activity of the glycosomal enzymes is included in the model (compare with Figure 3).

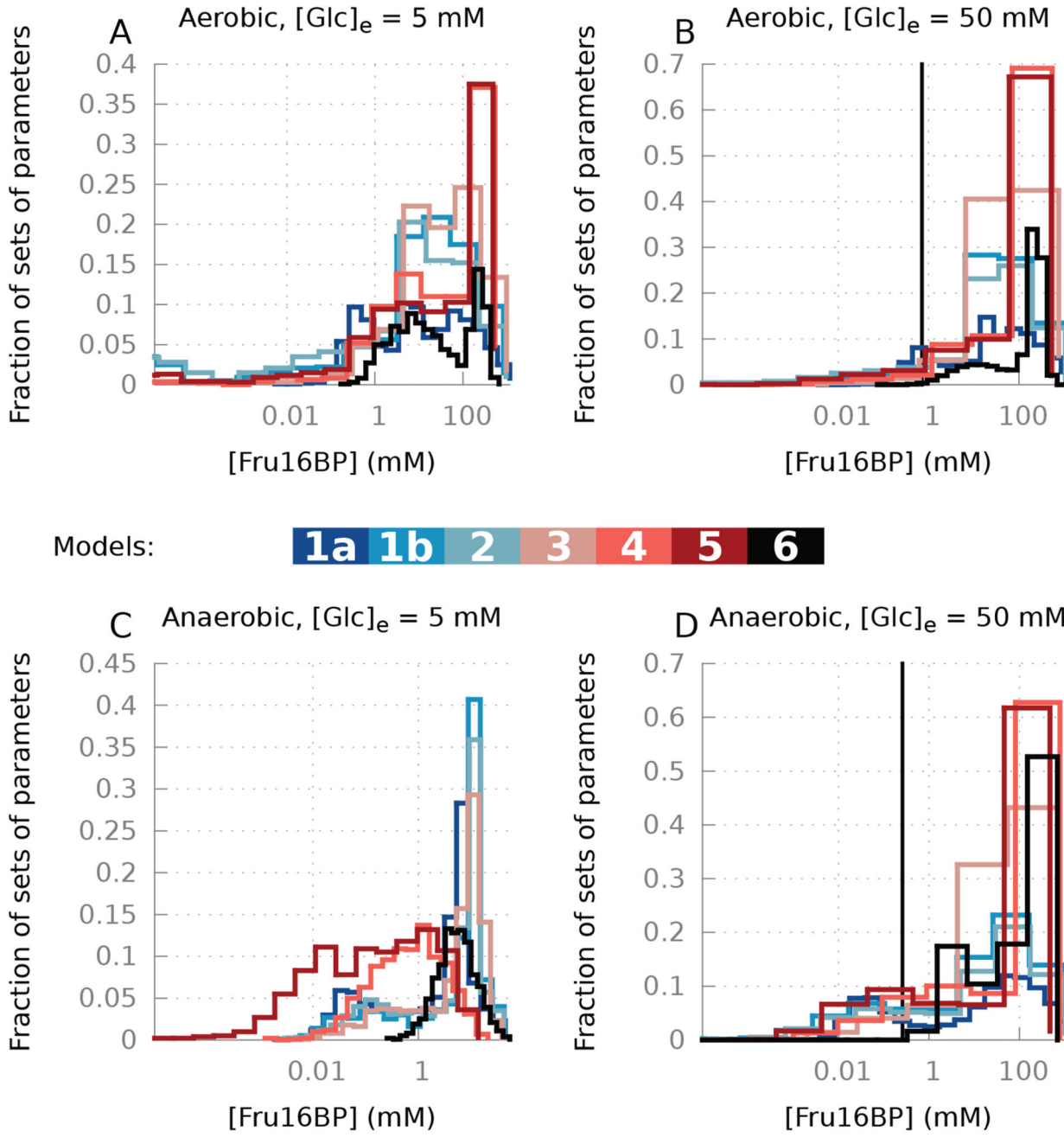

Figure 6.

Fructose 1,6-bisphosphate concentration in models with increasing glycosome permeability and cytosolic activities of the glycosomal enzymes. The distributions shown include all sampled fractions of cytocolic activities of the glycosomal enzymes for each model topology. (A, B) Aerobic conditions (−SHAM); (C,D) Anaerobic-like conditions (+SHAM). (A, C) 5 mM of external glucose; (B,D) 50 mM of external glucose; the vertical grey lines indicate the average whole-cell concentration of fructose 1,6-bisphosphate as measured by Visser and Opperdoes *et al.* [31]. When a fraction of cytosolic activity of the glycosomal

enzymes is included in the models, all model topologies have parameter sets that lead to the accumulation of high concentrations of fructose 1,6-bisphosphate. Hence, if glycosomal enzymes have a fraction of activity in the cytosol, it is probably regulated.

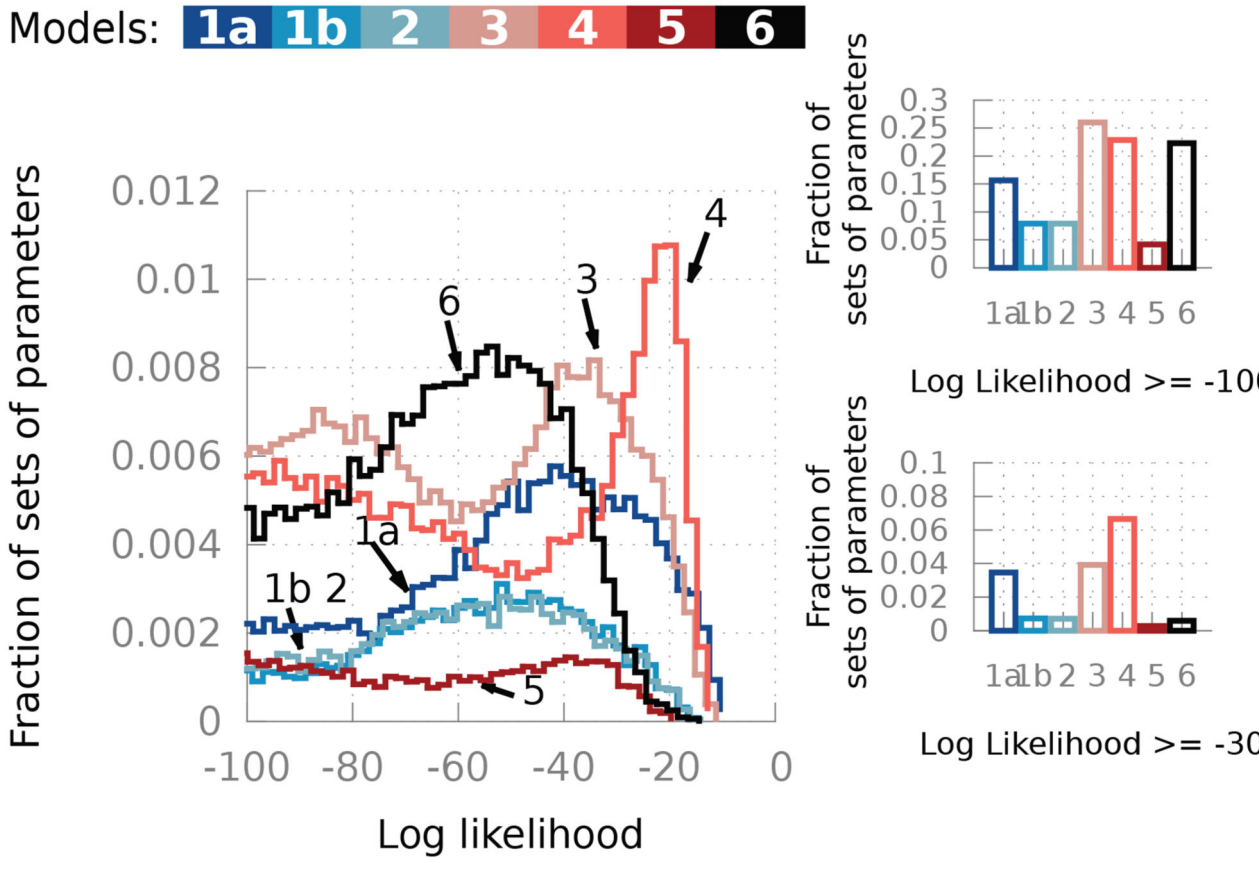

Figure 7.

Log-likelihood of models with increasing glycosome permeability and cytosolic activities of the glycosomal enzymes. The distributions shown include all sampled fractions of cytocolic activities of the glycosomal enzymes for each model topology. Inserts on the right show the fraction of parameter sets leading to a log-likelihood higher than −100 (top) higher than −30 (bottom) for each model topology. The best parameter sets produces the largest log-likelihoods (closer to 0), hence the models producing the closest match to experimental data are model 1a (impermeable with antiporter), 3 (permeable up to fructose 6-phosphate) and 4 (permeable up to fructose 1,6-bisphosphate).

Table 1

Fold-change of the median maximal activities significantly different in the best parameter sets (highest 1% log-likelihood) when compared to the other sampled values

Columns represents the different model topologies. See Supplementary Table S1 for all results. The fold-changes are computed as the median value of the best sets over the median value of all sets. When the fold-change is smaller then 1 (the parameter values are smaller in the best sets as compared to all sets) then −1/Fold-change is displayed. The value is missing when the difference between the best parameter values and all parameter values is not statistically significant (Mann-Whitney U test, see Methods).

Reaction	1a	1b	2	3	4	5	6
PGAMc	1.1	1.0	1.1	1.1	1.1	1.1	1.0
GlcTc	−1.1	−1.1	−1.1	−1.2	−1.2	−1.2	−1.2
GlyTc	1.3	1.1	1.1	1.2	1.2	1.1	1.2
GKg	1.2	1.1	1.1	1.1	1.1	1.1	1.1
ALDc	4.5	9.0	14.4	9.4			−2.0
HXKc	−3.5	−1.5	3.0	4.1		14.4	−1.5
PGKc	2.3		−1.8	4.6	2.3	11.9	−1.8
GAPDHc	1.2	−1.1	−1.1	1.1	1.1		−1.2
TPIc	1.9	−1.6		−1.5	−1.4		−2.8